The greatest flexibility with regard to choice of stationary phase, particle size, layer thickness, and chamber type is provided by RPC. Because of the availability of suitable vapour phases and combination of development modes, RPC offers the greatest separating power both in terms of the amount of sample and number of compounds to be separated.

It can be stated that PPC covers a special range of preparative separations. PPC does not compete with column liquid chromatography for purification and isolation of compounds from a complex matrix. Instead, the two approaches are complementary and together they enable successful and rapid separation. It is expected that as a result of development of modern forced-flow and multiple-development techniques, PPC will further expand its importance in the isolation and purification of natural and synthetic products.

See also: **II/Chromatography: Liquid:** Large-Scale Liquid Chromatography. **Chromatography: Thin-Layer (Planar):** Densitometry and Image Analysis; Instrumentation; Modes of Development: Conventional; Modes of Development: Forced Flow, Overpressured Layer Chromatography and Centrifugal; Spray Reagents.

Further Reading

Geiss F (1987) *Fundamentals of Thin Layer Chromatography* (*Planar Chromatography*). Heidelberg: Hüthig.

Hostettmann K, Hostettmann M and Marston A (1996) *Preparative Chromatography Techniques*, *Applications in Natural Product Isolation*. Berlin: Springer.

Nyiredy Sz (1995) Preparative layer chromatography. In: Sherma J and Fried B (eds) *Handbook of Thin-Layer Chromatography*. New York: Dekker, pp. 307–340.

Nyiredy Sz (1992) Planar chromatography. In: Heftmann E (ed.) *Chromatography*. 5th edn. Amsterdam: Elsevier, pp. A109–A150.

Nyiredy Sz, Erdelmeier CAJ and Sticher O (1986) Instrumental preparative planar chromatography. In: Kaiser RE (ed.) *Planar Chromatography*. Heidelberg: Hüthig, pp. 119–164.

Nyiredy Sz, Erdelmeier CAJ, Dallenbach-Toelke K, Nyiredy-Mikita K and Sticher O (1986) Preparative on-line overpressured layer chromatography (OPLC): A new separation technique for natural products. *Journal of Natural Products* 49: 885.

Nyiredy Sz, Botz L and Sticher O (1989) ROTACHROM®: A new instrument for rotation planar chromatography (RPC). *Journal of Planar Chromatography* 2: 53–61.

Nyiredy Sz, Dallenbach-Tölke K and Sticher O (1988) The 'PRISMA' optimization system in planar chromatography. *Journal of Planar Chromatography* 1: 336–342.

Poole CF (1992) *Chromatography Today*. Amsterdam: Elsevier.

Szabady B and Nyiredy Sz (1995) The versatility of multiple development in planar chromatography. In: Kaiser RE (ed.) *Modern TLC*. Düsseldorf: Verlag Chemie. pp. 345–367.

Sherma J and Fried B (1995) *Handbook of Thin-Layer Chromatography*. New York: Dekker.

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Radioactivity Detection

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Introduction

Thin-layer chromatography (TLC) is a technique which has been applied to a wide range of chemicals since its introduction in the early 1950s. The only limitation to its use is that a suitable method of detection must be available; however, this limitation is removed when the compounds of interest are radiolabelled. Nevertheless, since the introduction of thin-layer radiochromatography (TLRC), one major drawback in gaining widespread acceptance has been the lack of an easy method to quantify the distribution of radioactivity whilst still maintaining good resolution. The available detection methods have either been very time-consuming (e.g. autoradiography) or labour-intensive (e.g. zonal analysis) or could not match the resolution of the TLC separation itself. Over the years TLRC detectors have evolved and significantly improved, starting with scanners in the 1960s, followed by linear analysers in the 1980s and now the new 1990s generation of bioimaging analysers and InstantImager. The limitation of the scanners and linear analysers is that their resolution is lower than can be achieved by TLC itself. New detector technology such as phosphor imaging will lead to a renaissance in the use of TLRC due to the excellent resolution.

Detection and Measurement

The principal methods for detecting and quantifying radioactivity on TLC plates are autoradiography, zonal analysis (plate scraping followed by liquid scintillation counting) and direct measurement using radiation detectors. The method employed for

analysis depends on the available equipment, which generally depends on the amount of money available, and the type of experiment and information required. The various detection methods are discussed below and the technical descriptions in the present review provide information relating to the state-of-the-art modern-day detectors.

Autoradiography

Autoradiography is a detection method in which X-ray or photographic film is exposed to emissions from radioisotopes on TLC plates to produce an image on the film. After exposure (exposure time depends on the amount of radioactivity per zone), the film is developed to reveal the location of the areas of radioactivity as darkened spots or zones of varying optical density. The density is related to the amount of radioactivity in the spot/zone. Quantification can be done either by densitometry using a calibration curve produced by exposure to radioactive standards or by removing the areas of radioactivity (scraping/cutting) and counting them by liquid scintillation. The three principal exposure methods are direct exposure (autoradiography), direct exposure with an intensifying screen and fluorographic exposure (fluorography). The approximate minimum amounts of radioactivity that are required to give a suitable image with a 24 h exposure are shown in **Table 1** for the three different exposure methods.

Detection by direct exposure autoradiography involves intimate contact of the TLC plate with a photographic or X-ray film. Direct exposure is useful for all of the β-emitters, with the possible exception of low-level tritium-labelled samples. To improve the detection efficiency for γ-emitting e.g. ^{125}I) and high energy β-emitting isotopes (e.g. ^{32}P), the plates are exposed with intensifying screens placed behind the film. Commercially available intensifying screens consist of plates coated with inorganic phosphors. The fraction of radiation that passes completely through the film is absorbed by the phosphor, which in turn emits light that produces additional exposure of the film. The enhancement in sensitivity using an intensifying screen with preexposed film (see preflashing below) is of the order of 7–10-fold for ^{32}P and 16-fold for ^{125}I when compared to direct exposure without the screen.

Weak β-emitting isotopes (e.g. ^{3}H), adsorbed on TLC adsorbents, are inefficiently detected by direct exposure to X-ray films. The principal reasons for this inefficiency are the low energy and short range of the β-emissions and the barrier imposed by the protective coating of the X-ray film. To increase the sensitivity for these isotopes, a technique termed fluorography is employed. Fluorography involves the overcoating or impregnation of a scintillator into the TLC plate followed by direct exposure of the treated plate to the X-ray film. The scintillant, being in direct contact with the isotope, emits light when activated by the β-emission and exposes the film photographically. For efficient detection, the spectral sensitivity of the film should be matched to the wavelengths of light emitted by the scintillator. The scintillants can be incorporated by mixing with the adsorbent during preparation of the TLC plate or applied after development. Fluorographic reagents, such as 2,5-diphenyloxazole (PPO), can be added by spraying or dipping the plates.

The sensitivity of the technique can be further improved by lowering the exposure temperature and pretreating the film by partially exposing the film to a controlled flash of light (preflashing) before exposure to the radioactive sample. Preexposure to a flash of light greatly increases sensitivity and corrects the nonlinear response at low exposure levels. For maximum enhancement in sensitivity, both preflashing and cooling to temperatures between -70 and -80°C are utilized.

Zonal Analysis

The basic procedure involves removing areas of chromatographic adsorbent from a TLC plate

Table 1 Approximate lower detection limits on TLC plates for various exposure methods (dpm cm^{-2} with a 24-h exposure)

Exposure method	*^{3}H*	*^{14}C*	*^{32}P*	*^{125}I*
Direct exposure (autoradiography)	$2.6–13 \times 10^{5}$ [a]	220–650 [a]	500 [d]	1600 [d]
Direct exposure with intensifying screen			50 [d,e]	100 [d,e]
Fluorographic exposure (fluorography)	$2.0–6.6 \times 10^{3}$ [b,c]	50–450 [b,c]		

[a] Average range for direct exposure of film at temperatures between -78.5 and 25°C.
[b] Treated with a 7% solution of 2,5-diphenyloxazole (PPO) in diethyl ether and exposed at a temperature of -78°C.
[c] Treated with a mixture of 0.5% 2,5-diphenyloxazole (PPO) in methyl anthranilate at -80°C with Kodak X-OMAT AR film.
[d] Exposed at a temperature of -78°C.
[e] Preexposed Kodak X-OMAT R film with a calcium tungstate X-ray intensifying film. (Reproduced with permission from Clark and Klein, 1996.)

followed by measuring the associated radioactivity with each spot or zone. The zones are removed either by scraping the adsorbent from the plate (plate scraping) or by cutting pieces from flexible-backed plates and transferring the segments into counting vials. In an alternative procedure, which allows isolation of the radiolabelled sample, the plates are segmented and the radioactive components are eluted from the adsorbent with solvents and counted. To ensure maximum recovery of radioactivity by elution with solvent, the adsorbent should first be crushed to a fine powder. Measurement of radioactivity is generally accomplished using a liquid scintillation counter for the weak β-emitters. For the γ-emitters, the sectioned zones are counted without further sample preparation in an appropriate γ-counter.

This technique is relatively sensitive and provides quantitative detection for samples containing low levels of radioactivity. Single peaks containing 100 d.p.m. can be readily detected. Zonal analysis has been reported to be both as sensitive and specific as gas chromatographic-mass spectrometric analysis in the assay of [^{14}C]-labelled clinical samples. When the radiochromatograms are cut into sections and quantified using a γ-counter for the analysis of γ-emitting isotopes, the method is as precise as TLC scanning.

Radiation Detectors

Over the last 30 years or so the detection of radioactivity on TLC plates has taken dramatic leaps forward. Prior to the introduction of radiation detectors, the classical method used for the detection and quantification of radioactivity on a plate involved firstly exposure to X-ray film. This could take from a few hours up to 1–2 months and this technique only located the radioactivity. The second step after location was quantification which was achieved by removing the zone of interest, either by scraping off the silica gel or by cutting away if the plates were aluminium or plastic, followed by liquid scintillation counting. Such a procedure is extremely labour-intensive and is limited in terms of accuracy and resolution (see above).

The first radiation detectors were called radioscanners and these were developed and introduced in the early 1960s. This was a major step forward in the automatic detection and subsequent quantification of radioactive components on TLC plates. The sensitivity and resolution of the instruments were not very high but peaks could be detected and their relative amounts subsequently quantified. At around the same time, spark chambers were also developed for use with TLC plates. Although, these detectors could locate individual components on TLC plates, quantification was not possible.

Another major step forward for radio-TLC came in the early 1980s when the so-called linear analyser was introduced. This instrument was easier to use and more sensitive than the old scanners and was automated to the extent that up to four plates could be run overnight. As a consequence, improved quantitative results were obtained and analysis time was shortened. However, resolution was still not as good as that obtained by using autoradiography and two-dimensional plates could not easily be evaluated.

Currently there are a number of instruments available which have equal resolution to that obtained with autoradiography or are at least approaching it. These instruments include those using the new phosphor imaging technology, the multi-wire system, or the multi-detector system (micro-channel array detector).

The basic functioning of all these detectors is outlined below and a comparison of the advantages and disadvantages of each detector is given later.

Spark chambers The spark chamber is an easy-to-use, low cost technique for photographically locating areas of radioactivity on TLC plates. Exposure times are relatively short and the images obtained on Polaroid film can be quickly transferred back to the original chromatogram using an inbuilt episcope print projector. This means that the areas of radioactivity can then be removed for efficient counting using a liquid scintillation counter. The spark chamber can also be used for the rapid qualitative screening of plant and tissue sections to assess the degree of uptake.

Reviews of spark chambers and their uses have been published previously. Essentially, the spark chamber consists of electrodes contained in a chamber filled with a mixture of argon containing 10% methane, and this gives a high sensitivity to β-radiation. The gas is ionized by radioactive emissions and these emissions are recorded on film with a camera. The Polaroid film integrates the individual flashes produced over a suitable exposure period. Due to the intensity of the sparks the film is rapidly saturated, leading to blackening of the film, and hence direct quantification is not possible.

Radioscanners These instruments were developed and first sold commercially in the early 1960s and utilize a mechanically driven windowless gas-flow Geiger counter. These counters have an interchangeable aperture plate (collimator slit) which controls the size of the area being measured. The TLC plate is scanned by the moving detector head and the signal obtained from the radioactivity source is amplified and recorded. The resultant chromatogram can then

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be printed on a suitable recorder or integrator-plotter. When the speed of the scanner and recorder are synchronized the exact location of the radioactivity on the TLC plate can be obtained by aligning the chromatogram with the TLC plate. Some manufacturers continue to produce radioscanners but, due to the increasing number of new detection systems (described below) which have better sensitivity and resolution, the number of radioscanners available for quantitative TLC has decreased.

Linear analysers The introduction of the linear analyser provided a great boost for the users of radio-TLC since these detectors brought with them not only improved sensitivity and resolution but also much-improved automation. For example, up to four plates, each with several tracks, can be measured overnight and the chromatograms and accompanying quantitative tables automatically printed out. For the first time in this field the resultant data can be stored and reprocessed at a later date. With the development of new desktop publishing software, the chromatograms and quantitative results can be directly transferred into reports or publications.

The linear analysers currently used are based on imaging counters developed for high energy physics and medical imaging in the late 1960s and early 1970s. Essentially, the detector head moves automatically to any track on the TLC plate. Once in position the head is gently lowered on to the surface of this track of the TLC plate and the instrument is then ready to begin measurement. At this point the detector has formed a counting chamber since the TLC plate itself has closed the opening of the detector, making the counting chamber gastight. Immediately the detector is resting on the plate the flow of counting gas (argon/methane) is automatically activated and within a few seconds the counting chamber is purged of air and filled with the counting gas.

There are two kinds of systems available today which function in a similar way: each utilizes a different design to locate the exact position of the radioactivity on the plate. One system uses the resistive anode technique and a schematic diagram of this detector is shown in **Figure 1**. High voltage is applied to a 25 cm anode wire fixed along the length of a windowless detector (1 cm wide) and positioned directly above the TLC plate. This wire is constructed of carbon-coated quartz and has a high electrical resistance. When a radioactive emission enters the detector, the gas is ionized and electrons are produced along the particle track. The free electrons are accelerated towards the anode wire by the electric field produced by the high voltage. The electrons continue to ionize more gas as they approach the wire, and the resulting number of electrons becomes large enough to be detected electronically. The pulse of electrons is collected on the anode wire near the position of the initial ionization. The charge divides in the wire, and pulses appear in the amplifiers located at both ends of the wire. The amplitude of the pulse measured by each amplifier is proportional to the resistance between that end of the wire and the position where the electrons were collected. The ratio of these two pulses is linearly related to the original position of the event on the wire. The position of each event is calculated and stored in a computer memory to provide a digital image of the distribution of radioactivity on the plate.

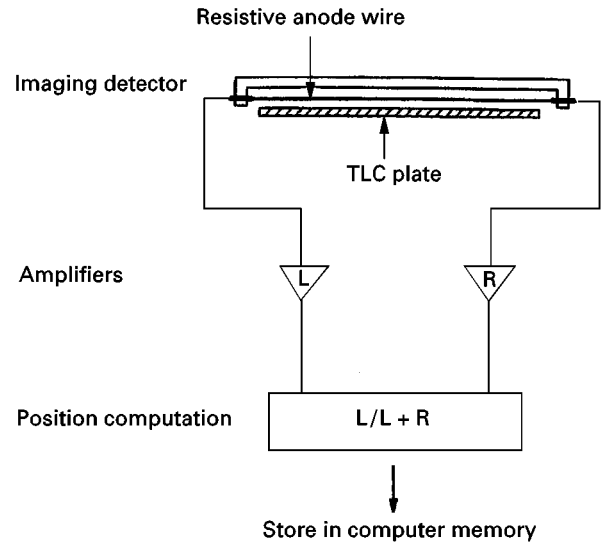

Figure 1 Schematic diagram of a linear analyser detector with a resistive anode wire. (Reproduced with permission from Clark and Klein, 1996.)

The second type of detection system uses the delay wire technique; a schematic diagram of this detector is shown in **Figure 2**.

The β-radiation (fast electrons) emitted from the radioactive source on the plate ionizes the counting gas which has been specifically chosen so that this process can freely take place. This is the primary mode of ionization and the resultant charged particles, free electrons and positive ions, are then accelerated towards the anode wire and cathode, respectively. In this primary mode of ionization the free electrons are accelerated to such an extent that they themselves cause ionization of the counting gas, producing further free electrons and ions and this is the secondary ionization mode. This continues, causing an avalanche of ions from the primary point of ionization towards the anode wire.

Concurrently, the positive ions produced move relatively slowly towards the cathode. These positive

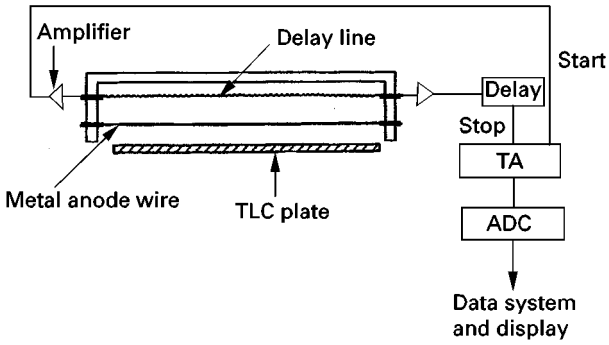

Figure 2 Schematic diagram of a linear analyser detector utilizing a delay wire technique. TA, Time-to-amplitude; ADC, analog-to-digital converter. (Reproduced with permission from Clark and Klein, 1996.)

ions sometimes combine with electrons, producing ultraviolet radiation of sufficient energy to cause further ionizations in a process known as the photoelectric effect. Once sufficient ionization has taken place, a spark is produced, which gives rise to a pulse in both the anode and cathode. The amplitude of the pulse is proportional to the number of ions produced and hence this type of detector is generally called a proportional counter.

The above is a description of the principle of detection. The location of the source of the ionizations is obtained by making use of a delay wire. The delay wire is a very thin wire which is wound over the cathode and pulses pass along this wire in both directions. The pulses are detected by amplifiers at each end of the wire. The arrival of a pulse at one end starts the time-to-amplitude (TA) circuit, while the other pulse is delayed and provides a stop signal in the circuit. The difference between the time of arrival at the two ends of the wire can thus be measured and is proportional to the position of the initial ionization. An analog-to-digital converter (ADC) converts the TA signal to a digital position value that is processed by the data system.

Using this method of detection, the whole of the delay line remains active and thereby the entire length of the chromatogram can be measured at the same time. Once one track of a TLC plate has been measured according to the pre-set time, it automatically moves to the next and the measuring process is repeated.

Radioanalytic imaging system (Ambis) When this instrument was introduced in about 1988, a description of its functioning was reported. The Ambis 4000 directly detects β-particles from a wide variety of isotopes and is suitable for gels, blots, TLC plates and any sample type of the dimensions 20×20 cm. It is reported in the company literature that this instrument can be 100 times faster than X-ray film.

The detector consists of 3696 individual detector elements (each giving a data point) configured in a hexagonal array. Image quality is improved by increasing the number of data points and this is achieved by moving the sample through 72, 144 or 288 discrete positions. Therefore, counts are recorded in 266 112, 532 224 or 1 064 448 data points (i.e. 3696 × number of discrete positions) from which an image is obtained. This image can then be displayed on a monitor and the areas of radioactivity quantified. A background detector which operates concurrently and in a similar way is located above the main detector, and compensates for background radiation.

Different resolution plates, which have different size and shape apertures, can be inserted into the instrument and these plates control the resolution and efficiency (i.e. sensitivity) of the instrument. In general, this means that, using the correct aperture, the detector can be tuned to obtain maximum resolution (at the expense of sensitivity). Conversely, when the instrument is tuned for maximum sensitivity, this is at the expense of resolution. Therefore, aperture choice is governed by sample size and the number and resolution of components required within the sample.

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Multiwire Proportional Counters (MWPC)

Digital Autoradiograph (Berthold) This two-dimensional detector is reported to be 100 times more sensitive than the linear analyser and measures all areas of radiation from a 20×20 cm surface simultaneously.

The radio-TLC plate is placed on the measuring table and is then automatically loaded into the detector, which also controls the flow of the P-10 counting gas (90% argon + 10% methane). The detector is principally a two-dimensional position-sensitive MWPC. Essentially, it consists of three parallel wire planes, *X*, *Y* and *Z*, each with 100 wires. The spacing between the planes and the wires is only a few millimetres. The central plane (*Z*) is maintained at a positive potential of 1800 V and the counting chamber is filled with P-10 gas. Charged pulses are generated on the *Z* plane wires by ionizing particles (β-particles). The orthogonally crossed wire planes *X* and *Y*, below and above *Z*, pick up the charge signals from the *Z* plane at their position of origin, hence the position of the radioactivity on the TLC plate can be located.

The signals from the wire planes are transmitted via preamplifiers, pulse shapers, discriminators and logic circuits to ADC which are finally coupled to a data acquisition system.

InstantImager (*Canberra Packard*) This microchannel array detector provides direct electronic detection and real-time imaging of radioactivity on flat surfaces such as gels, blots, tissue slices and, of course, TLC plates. The detector consists of an array of 210 420 so-called microchannels (diameter 400 μm) in a 20 × 24 cm multilayer plate. The microchannel array plate is a laminated surface about 3 mm thick with alternating conductive and nonconductive materials. A voltage step gradient is applied to the successive conductive layers to create a high electric field (approximately 600 volts mm^{-1}) in the microchannels. The β-particle emitted from the radioactive source ionizes a gas (argon with small amounts of carbon dioxide and iso-butane) in one of the microchannels. The electrons produced are accelerated by the high electric field in the microchannel, further ionizing the gas, resulting in a cloud of electrons. In this way the microchannels serve as both collimators and preamplifiers.

The cloud of electrons migrates up an electric field gradient into a multiwire chamber located on top of the multilayer. This chamber consists of an anode plane of thin anode wires and two cathode planes (*X* and *Y*), as described above for the Digital Autoradiograph. Further avalanche amplification occurs, resulting in electric pulses in the *X* and *Y* cathode tracks. The resultant signals are digitized and then decoded to identify the microchannel in which the primary ionization took place, hence locating the position of the radioactive emission. A schematic representation of the microchannel detector is shown in **Figure 3**.

BioImaging/phosphor imaging analysers The phosphor imagers make use of an imaging plate which is a two-dimensional sensor formed by a layer of fine crystals of photostimulable phosphor (BaFBr : Eu^{2+}). The emitted β-energy is stored upon exposure. In the reading unit the imaging plate is scanned with a laser beam. The energy of the laser stimulates the stored

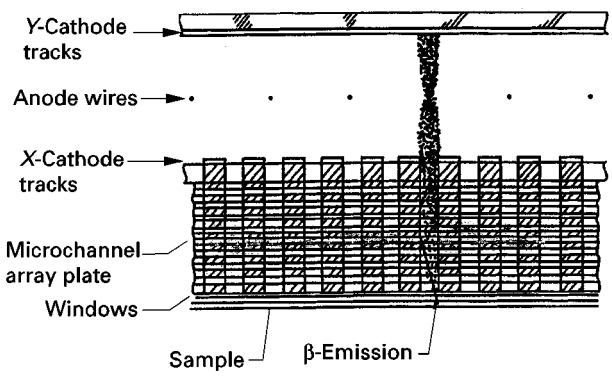

Figure 3 Schematic diagram of the microchannels of the InstantImager. (Courtesy of David Englert, Canberra Packard, Meriden, CT, USA.)

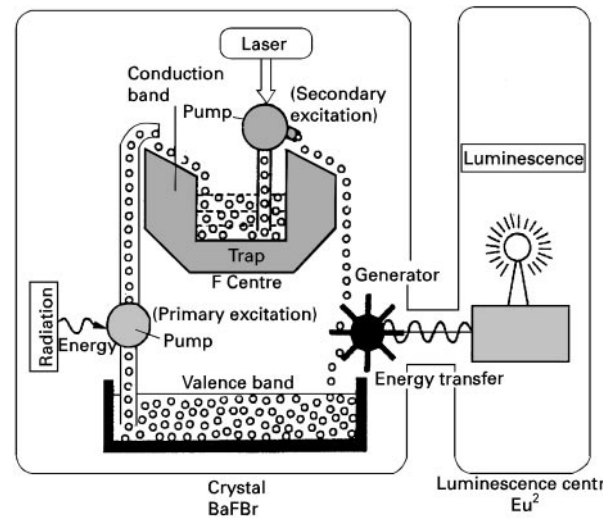

Figure 4 Schematic diagram of the principle of detection of the PhosphoImaging analyser. (Courtesy of Fuji Photo Film Co. Ltd, Tokyo, Japan.)

electrons to return to the ground state and to emit luminescence in proportion to the recorded radiation intensity. This luminescence is collected in a photomultiplier tube and converted into an electrical signal. A schematic diagram of the principle of detection is shown in **Figure 4**.

Data recording and analysis are carried out at a workstation. After reading, the image data on the imaging plate can be erased by exposure to incandescent light and thus the plate can be reused. Imaging plates for the normal weak β-emitters are available and a specially designed plate for tritium is available. An illustration of the whole imaging process is given in **Figure 5**.

A prerequisite for good results is to expose the plates in a lead shielding box, particularly those that require longer than 1–2 h exposure time. In this way the contribution of natural background radiation is reduced.

Over the last few years there has been a significant expansion in the variety of instruments available and in the type of imaging plates on offer. Instrumentation has been improved and targeted as far as applications are concerned. For instance, Fuji now has six Phosphor Imaging plate scanners (BAS 1000, 1500, 2000, 2500, 5000 and the new 1800) and Canberra Packard has brought out the Cyclone. Fuji also now offers the FLA 2000 which combines fluorescent and radioisotope detection. The major improvement in the BAS range of instruments has been in resolution, whereby the BAS 5000 can now operate with a resolution of 25 μm, although when used at this high resolution the storage memory required for each scan is extremely high.

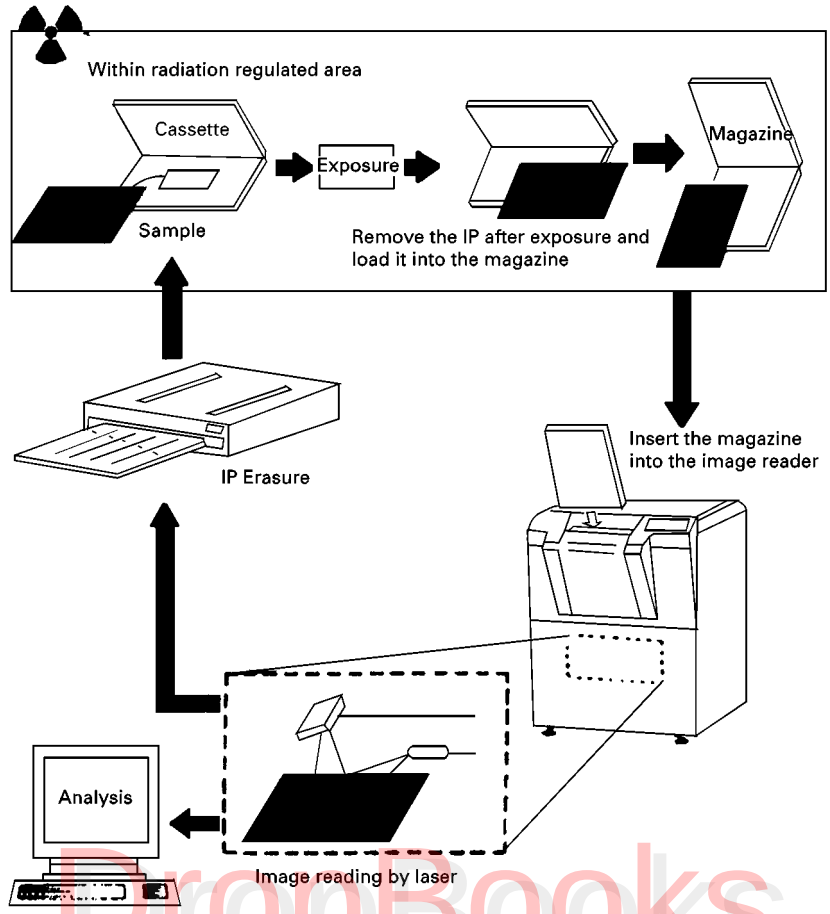

Figure 5 Illustration of the phosphoimaging process. IP, Imaging plate. (Courtesy of Fuji Photo Film Co. Ltd, Tokyo, Japan.)

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A further instrument using similar technology, recently introduced by Packard, is the Cyclone™. In this instrument, a solid-state diode laser and confocal optical system moves down the storage phosphor screen as the screen rotates on a carousel. In this process the laser excitation and light collection optics remain in a fixed position relative to the screen surface, so that laser bleed associated with other detectors is eliminated. Furthermore, light collection is increased compared to that obtained with fibreoptic bundles.

A range of imaging plates is now available and these should be chosen according to instrument and requirement. Currently, Fuji is the leading supplier and offers the BAS III, MP, SR, TR and ND imaging plates: care must be taken when selecting a plate because not all plates can be used with all instruments. A range of cassette sizes is also available from Fuji according to plate size.

Comparison of TLRC Detection Methods

As described at the beginning of this article, there are three principal techniques for the analysis of radioactive components on TLC plates, autoradiography, zonal analysis and mechanical detectors (e.g. linear analysers, phosphor imagers, MWPC detectors). The technique of choice depends on a number of parameters but of primary consideration are sensitivity and resolution. Other parameters to be considered are quantification, linear dynamic range, speed, sample throughput and preservation of sample. A comparative summary of the detection methods with respect to these parameters is shown in **Table 2**.

Both autoradiography and zonal analysis have a number of drawbacks, including sensitivity and resolution, but primarily both techniques are extremely time-consuming. Linear analysers offer a good compromise between speed, resolution and quantitative accuracy. However, the performance of the linear analysers falls well below that of the currently available MWPC detectors and phosphor imagers in all respects. Sensitivity, quantification and, particularly, resolution are significantly superior, resulting generally in much better quality chromatograms. The phosphor imagers have slightly better resolution than the MWPC detectors but the disadvantage of phosphor imagers is that the chromato-

Table 2 Comparison of thin-layer radiochromatographic analysis techniques

Parameters	*Autoradiography*	*Zonal analysis*	*Linear analyser*	*MWPC detector*	*Phosphor imager*
Sensitivity	+	+++++	++	+++++	+++++
Resolution	+++++	+	++	++++	+++++
Quantification	+	+++++	++++	+++++	+++++
Dynamic range	+++	+++	+++	+++++	+++++
Speed	+	++	+++++	+++++	++++
Sample throughput	+	+	+++	++++	+++++
Preserves sample	Yes	No	Yes	Yes	Yes

+++++, Excellent; ++++, very good; +++, good; ++, satisfactory; +, poor. (Reproduced with permission from Clark and Klein, 1996.)

gram development cannot be seen in real time. Also, for a single plate the analysis time with the MWPC detectors is quicker but when more sample throughput is required, then the phosphor imagers have the advantage since many plates can be exposed simultaneously.

In general, as the newer range of detectors were brought on to the market they were very expensive in comparison to the linear analysers. However, with increasing competition and technological developments, prices are slowly coming down.

Future Developments in TLRC

The new range of detectors have significantly improved sensitivity and resolution; most have a resolution of under 1 mm and the phosphor imagers are able to obtain a resolution of as low as 0.025 mm. Also, current detectors are now able to detect spots of radioactivity on a plate containing less than 10 d.p.m. in a relatively short period of time. As sensitivity and resolution are continually improving, the future major development of TLRC probably lies in the realm of full automation using robots, from the application of multiple samples to the TLC plate, to development and drying, transport to the detector, measurement and finally printing of the chromatogram and quantitative results.

Further Reading

Clark T and Klein O (1996) Thin-layer radiochromatography. In: Sherma J and Fried B (eds) *Handbook of Thin Layer Chromatography*, 2nd edn. New York: Marcel Dekker.

Filthuth H (1982) Radioscanning of TLC. In: Touchstone JC (ed.) *Advances in Thin Layer Chromatography*, pp. 89–123. New York: John Wiley.

Filthuth H (1989a) New detector for radiochromatography and radio-labelled multisample distributions. The digital autoradiograph. *Journal of Planar Chromatography* 2: 198.

Filthuth H (1989b) Detection of radioactivity distribution with position-sensitive detectors, linear analyzer, and digital autoradiograph. In: Touchstone JC (ed.) *Planar Chromatography in the Life Sciences*, p. 167. New York: John Wiley.

Hamaoka T (1990) Autoradiography of new era replacing traditional X-ray film bio-imaging analyzer BAS2000. *Cell Technology* 9: 456.

Johnston RF, Pickett SC and Barker DL (1990) Autoradiography using storage phosphor technology. *Electrophoresis* 11: 355.

Klein O and Clark T (1993) The advantages of a new bio-imaging analyzer for investigation of the metabolism of ^{14}C-radiolabelled pesticides. *Journal of Planar Chromatography* 6: 369.

Miyahara J (1989) Visualising things never seen before. The imaging plate: a new radiation image sensor. *Chemistry Today* 223: 29.

Nakajima E (1993) Radioluminography, a new method for quantitative autoradiography in drug metabolism studies. *Radioisotopes* 42: 228.

Prydz S (1973) Summary of the state of the art in radio-chromatography. *Analytical Chemistry* 45: 2317.

Roberts TR (1978) *Radiochromatography, The Chromatography and Electrophoresis of Radiolabelled Compounds*, pp. 45–83. Amsterdam: Elsevier.

Shulman SD (1982) Quantitative analysis by imaging radiation detection. In: Touchstone JC (ed.) *Advances in Thin Layer Chromatography*, pp. 125–137. New York: John Wiley.